# Effects of Sonneratia apetala Fruit Extract on Learning and Memory in Aged Mice and Its Mechanism: Postprint

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**Date:** 2018-10-26T00:00:00+00:00

## Abstract

Sonneratia apetala fruit is the fruit of the true mangrove Sonneratia apetala. This study investigated the effects of different extracts of Sonneratia apetala fruit on the learning and memory abilities of D-galactose-induced aging mice and their underlying mechanisms. The Morris water maze test was employed to assess the effects on learning and memory, HE staining was used to observe changes in cerebral neurons across groups, and the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), nitric oxide (NO) content, and monoamine oxidase (MAO) activity in brain tissue were measured. The results demonstrated that, compared with the model group, mice treated with different extracts of Sonneratia apetala fruit exhibited significantly shortened escape latency ($P<0.05$) and significantly increased time spent in the target quadrant ($P<0.05$) in the water maze test. Neuronal damage in the brain was markedly reduced in the treatment groups compared with the model group, with increased SOD and GSH-Px enzyme activities ($P<0.05$) and significantly decreased NO content and MAO activity in the brain ($P<0.05$). Different extracts of Sonneratia apetala fruit ameliorate the learning and memory impairments in D-galactose-induced aging mice by enhancing the activities of endogenous antioxidant enzymes (SOD, GSH-Px) and reducing NO content and MAO activity in the brain.

## Full Text

### Study on the Effect and Mechanism of Sonneratia apetala Fruit Extract on Learning and Memory in Aging Mice

**DOI:** 10.11931/guihaia.gxzw201807027

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## Abstract

This study investigated the effects and mechanisms of different extracts from Sonneratia apetala fruit on learning and memory in D-galactose-induced aging mice. The Morris water maze test was used to assess learning and memory performance, while hematoxylin-eosin (HE) staining was employed to observe neuronal changes in brain tissue. Additionally, brain tissue levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), nitric oxide (NO), and monoamine oxidase (MAO) were measured. Compared with the model group, mice treated with different S. apetala fruit extracts showed significantly shorter escape latencies ($P < 0.05$) and increased target quadrant dwell times ($P < 0.05$) in the water maze test. HE staining revealed significantly reduced neuronal damage in extract-treated mice compared to the model group. Furthermore, SOD and GSH-Px activities were significantly elevated ($P < 0.05$), while NO content and MAO activity were significantly decreased ($P < 0.05$) in the brain tissue of treated mice. These findings demonstrate that different extracts of S. apetala fruit can improve learning and memory in D-galactose-induced aging mice, likely by enhancing endogenous antioxidant enzyme activity (SOD, GSH-Px) and reducing NO content and MAO activity in the brain.

**Keywords:** Sonneratia apetala fruit; D-galactose; learning and memory; nerve cells; antioxidant activity

## Introduction

Aging is an inevitable biological process characterized by progressive decline in physiological functions, reduced adaptability, and diminished homeostatic capacity. One of the most prominent manifestations of aging is decreased antioxidant capacity (Wickens, 2001). During aging, increased free radicals and their induced harmful substances accumulate in the body while defense mechanisms weaken, leading to impaired antioxidant function (Zhang et al., 2002). The brain is particularly vulnerable to aging, undergoing structural changes including neuronal loss, brain atrophy, and reduced antioxidant capacity (Huang et al., 2010). Consequently, identifying potent antioxidant compounds represents a critical focus of anti-aging research.

Mangrove plants, which inhabit tropical and subtropical intertidal zones, constitute an important component of marine vegetation. Their unique habitat—characterized by high salinity, intense light exposure, and chronic root hypoxia—necessitates robust antioxidant defense systems. Consequently, most mangrove plant organs (roots, stems, leaves, flowers, and fruits) exhibit antioxidant activity. The n-butanol extracts of *Rhizophora apiculata* and *Acanthus ilicifolius* roots, rich in polyphenols and flavonoids, can inhibit DPPH and ABTS radicals and reduce oxidative stress in mouse brain (Asha et al., 2012). The n-butanol extract of *Rhizophora mangle* stems can modulate SOD, GPx, and GR activities while increasing GSH levels and decreasing LPO in mouse gastric tissue (de-Faria et al., 2012). Additionally, 12-deoxyphorbol 13-(3E,5E-decadienoate) isolated from *Excoecaria agallocha* stems can inhibit oxidative stress in rat brain cell membranes (Erickson et al., 1995).

*Sonneratia apetala*, a tree species belonging to the family Sonneratiaceae, is native to Bangladesh and was introduced to China in the 1980s. It is now widely distributed in Hainan, Guangxi, Guangdong, Fujian, and Taiwan provinces (Wang & Chen, 2002). The fruits of *S. apetala* are edible and can be used to produce fruit wine, containing abundant amino acids, total phenolics, and anthocyanins (Lin et al., 2009a; Lin et al., 2009b; Patra et al., 2015). Cao et al. (2015) isolated 16 compounds from fresh *S. apetala* fruits, including the novel compound ethyl n-butyl malate. In Bangladeshi folk medicine, the fruits are used to treat sprains (Ganguly & Sincar, 1974). Currently, *S. apetala* fruits in China remain underutilized, representing a significant waste of marine biological resources. To promote their development and utilization, we have conducted multi-level studies, previously demonstrating potent in vitro free radical scavenging capacity of *S. apetala* fruit extracts (Yi et al., 2017). To further verify their in vivo antioxidant activity, this study employed a D-galactose-induced aging model to investigate the effects of *S. apetala* fruit extracts on learning, memory, and antioxidant stress activity in aging mice, exploring the underlying mechanisms to provide an experimental basis for further development of this resource.

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### 1.1 Experimental Animals

One hundred eight female Kunming mice, weighing 18–22 g, were obtained from the Experimental Animal Center of Guangxi Medical University (License No. SCXK Gui 2014-0002). Animals were housed under standard laboratory conditions (temperature 23 °C, humidity 45–55%) with a 12-hour light/dark cycle and free access to food and water.

### 1.2 Preparation of *S. apetala* Fruit Extracts

Fresh fruits were collected from the Maowei Sea Mangrove Nature Reserve in Guangxi and authenticated as *Sonneratia apetala* by Engineer Xu Mingben from

the Guangxi Beibu Gulf Marine Research Center. The fruits were extracted with 95% ethanol under reduced pressure to obtain the ethanol extract (SEE). This extract was sequentially partitioned with ethyl acetate and n-butanol to yield the ethyl acetate extract (SEAE) and n-butanol extract (SBE), respectively. All extracts were concentrated under reduced pressure and lyophilized for subsequent use.

### 1.3 Main Reagents and Instruments

**Reagents:** D-galactose and water-soluble vitamin E (Beijing Solarbio Science & Technology Co., Ltd., Batch Nos. D8310 and ST9160); hematoxylin, eosin, and neutral resin (BIOSWAMP, Batch Nos. PAB180015, PAB180016, and PAB180017); SOD, GSH-Px, NO, and MAO assay kits (Nanjing Jiancheng Bioengineering Institute, Batch Nos. 20170214, 20170220, 20170224, and 20170213).

**Instruments:** Infinite 200 microplate reader (TECAN, Austria); ST16R high-speed refrigerated centrifuge (Thermo Fisher Scientific, Germany); HH-S6 digital water bath (Jintan Medical Instrument Factory); Cary 100 UV-Vis spectrophotometer (Varian, USA); DHG-9140A electric thermostatic drying oven (Shanghai Jinghong Laboratory Equipment Co., Ltd.); CX41 upright microscope (Olympus, Japan); RM2235 rotary microtome (Leica Microsystems); TB-718D paraffin embedding machine (Hubei Taive Technology Co., Ltd.).

### 1.4 Animal Grouping, Aging Model Establishment, and Administration

After 3–5 days of acclimatization, mice were randomly divided into nine groups (n = 12 each): normal control, model, positive control (water-soluble vitamin E, 100 mg/kg), SEE high-dose (SEE-H, 250 mg/kg), SEE low-dose (SEE-L, 125 mg/kg), SEAE high-dose (SEAE-H, 76 mg/kg), SEAE low-dose (SEAE-L, 38 mg/kg), SBE high-dose (SBE-H, 19 mg/kg), and SBE low-dose (SBE-L, 9.5 mg/kg). Except for the normal group, all mice received daily subcutaneous injections of D-galactose (120 mg/kg) for 13 weeks to induce aging. The normal group received equivalent volumes of normal saline. Administration via gavage began at week 8 and continued for 42 days. The normal and model groups received normal saline (20 ml/kg). At the end of the experiment, mice were sacrificed by cervical dislocation for sample collection.

### 1.5 Morris Water Maze Test

The Morris water maze test was conducted during the final five days of the experiment. Mice underwent four daily training sessions for the first four days, with each session starting from a different quadrant. Animals were placed in the water facing the pool wall, and the time to locate the platform (escape latency) was recorded within a 60-second limit. If a mouse failed to find the platform within 60 seconds, it was guided to the platform and allowed to remain for 10

seconds (escape latency recorded as 60 seconds). On day 5, a spatial probe test was performed with the platform removed, and the number of crossings over the original platform location was recorded as a measure of memory retention.

### 1.6 Morphological Observation of Brain Tissue

Two mice from each group were randomly selected and sacrificed. Brains were immediately removed on ice, rinsed with ice-cold saline to remove surface blood, blotted dry, and fixed in 4% paraformaldehyde. Paraffin-embedded sections were prepared and stained with HE for neuronal observation by Wuhan Hualianke Biotechnology Co., Ltd.

### 1.7 Tissue Preparation and Biochemical Assays

Mice were sacrificed by cervical dislocation, and brains were rapidly removed on ice and rinsed with ice-cold saline to remove blood. After blotting dry, brain tissue was homogenized in saline to prepare 10% tissue homogenates. SOD activity, GSH-Px activity, NO content, and MAO activity were measured according to the kit instructions.

### 1.8 Statistical Analysis

Data were analyzed using SPSS 20.0 software. One-way ANOVA followed by LSD test was used for inter-group comparisons. $P < 0.05$ was considered statistically significant. Data are expressed as mean ± standard error of the mean (SEM).

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## 2 Results

### 2.1 Effects on Learning and Memory Performance

As shown in Table 1 , compared with the normal group, the model group exhibited significantly prolonged escape latencies ($P < 0.01$) and reduced platform crossing times ($P < 0.01$), confirming successful establishment of the aging model with impaired learning and memory. Compared with the model group, SEE-H treatment significantly shortened escape latencies and increased platform crossing times ($P < 0.01$). Both SEAE-H and SEAE-L groups showed significantly shorter escape latencies ($P < 0.01$) and increased platform crossing times ($P < 0.01$), with effects comparable to the normal group.

**Table 1** Effects of *Sonneratia apetala* fruit extracts on escape latency and platform crossing frequency in D-galactose-induced aging mice ($\bar{x} \pm s$, $n = 12$)

| Group | Escape Latency (s) | Platform Crossing Times |
|---|---|---|
| | Day 1 | Day 2 |

| Group | Escape Latency (s) | Platform Crossing Times |
|---|---|---|
| Normal | 32.97±4.77 | 15.73±5.96 |
| Model | 49.56±11.23# | 48.87±6.65## |
| Positive | 39.46±11.24 | 21.74±7.99** |
| SEE-H | 42.86±8.88 | 29.03±13.01** |
| SEE-L | 33.41±8.87** | 35.69±8.12* |
| SEAE-H | 35.43±7.90* | 32.11±10.15** |
| SEAE-L | 39.92±4.71 | 22.70±6.31** |
| SBE-H | 34.31±6.94** | 32.78±10.16** |
| SBE-L | 31.26±8.72** | 23.79±10.88** |

Note: #P < 0.05, ##P < 0.01 vs. normal group; *P < 0.05, **P < 0.01 vs. model group.

### 2.2 Morphological Observations of Brain Tissue

Aging is associated with brain atrophy and neuronal death (Jiang et al., 2016). As shown in Figure 1 [Figure 1: see original paper], normal group mice exhibited compact neuronal arrangement with clear cellular structures. In contrast, the model group showed extensive deep staining and numerous necrotic neurons, indicating D-galactose-induced brain atrophy and necrosis. The positive control group displayed minimal necrotic cells with clear neuronal structures, demonstrating vitamin E's protective effects. SEE-H treatment resulted in few necrotic cells with clear structures, while SEE-L showed slight necrosis with compact cell arrangement, indicating dose-dependent neuroprotective effects. Both SEAE-H and SEAE-L groups showed clear neuronal structures with minimal necrosis, demonstrating effective prevention of age-related neuronal death. SBE-H treatment showed slightly enlarged intercellular spaces but clear cell outlines, while SBE-L exhibited minimal necrotic neurons with compact arrangement, confirming protective effects against D-galactose-induced brain atrophy.

**Figure 1** Morphological observation of brain tissue in each group (HE staining, 200×)

Note: A. Normal group; B. Model group; C. Positive group; D. SEE-H group; E. SEE-L group; F. SEAE-H group; G. SEAE-L group; H. SBE-H group; I. SBE-L group.

### 2.3 Antioxidant Indices in Brain Tissue

As shown in Table 2 , compared with the normal group, the model group exhibited significantly decreased SOD and GSH-Px activities (P < 0.01) and elevated NO content and MAO activity (P < 0.01), confirming successful model establishment with accumulated harmful substances and reduced antioxidant capacity. The positive control group showed significantly increased SOD and GSH-Px activities (P < 0.01) and decreased NO content and MAO activity (P < 0.01),

demonstrating vitamin E' s efficacy. Both SEE-H and SEE-L groups significantly increased SOD and GSH-Px activities ($P < 0.01$) and reduced MAO activity ($P < 0.01$), with SEE-H also significantly decreasing NO content ($P < 0.01$) and SEE-L showing a moderate reduction ($P < 0.05$). SEAE-H significantly reduced NO content and MAO activity ($P < 0.01$), while SEAE-L increased SOD activity ($P < 0.01$) and decreased both NO content and MAO activity ($P < 0.01$), with low-dose showing superior effects. SBE-H significantly elevated SOD and GSH-Px activities ($P < 0.01$) while reducing NO content and MAO activity ($P < 0.01$). SBE-L significantly decreased NO content and MAO activity ($P < 0.01$), demonstrating dose-dependent antioxidant enhancement.

**Table 2** Antioxidant indices in brain tissue of mice in each group ($\bar{x} \pm s$, $n = 12$)

| Group | SOD Activity (U/mg prot) | GSH-Px Activity (U/mg prot) | NO Content ( mol/g prot) | MAO Activity (U/mg prot) |
|---|---|---|---|---|
| Normal | 312.42±29.84 | 87.95±13.75 | 0.99±0.21 | 3.81±0.35 |
| Model | 195.24±15.63## | 50.65±9.25## | 1.47±0.45## | 8.80±0.99## |
| Positive | 416.52±52.59** | 91.72±24.69** | 0.80±0.15** | 1.89±1.17** |
| SEE-H | 269.90±39.34** | 86.18±9.82** | 0.86±0.12** | 2.68±0.86** |
| SEE-L | 292.40±36.25** | 82.04±24.09** | 1.11±0.76* | 2.72±0.58** |
| SEAE-H | 237.36±81.79 | 72.10±7.40 | 0.87±0.20** | 4.55±3.74** |
| SEAE-L | 354.76±64.62** | 65.79±40.76 | 0.69±0.19** | 2.00±0.98** |
| SBE-H | 334.98±51.88** | 86.82±28.64** | 0.72±0.16** | 2.78±0.92** |
| SBE-L | 227.22±67.20 | 53.71±16.36 | 0.47±0.13** | 1.10±1.07** |

Note: #$P < 0.05$, ##$P < 0.01$ vs. normal group; *$P < 0.05$, **$P < 0.01$ vs. model group.

## 3 Discussion and Conclusion

Aging is a spontaneous and inevitable biological process involving progressive degeneration of organ structure, decline in physiological and biochemical functions, and reduced adaptability, resistance, and homeostatic capacity. Various animal models have been used to study aging and dementia, with chronic D-galactose administration being a common method to simulate aging. Mice receiving long-term D-galactose injections exhibit dull and yellowish fur, sluggish

movement, and impaired learning and memory, accompanied by significantly reduced brain SOD and GSH-Px activities and elevated NO content and MAO activity. Water-soluble vitamin E significantly improved these indices, confirming successful model establishment.

During metabolism, organisms continuously generate free radicals that cause metabolic disorders, disease development, and accelerated aging, forming the basis of the free radical theory of aging. This theory posits that aging is associated with increased free radicals, elevated harmful substances, and oxidative imbalance due to declining defense mechanisms (Liu et al., 2016). SOD and GSH-Px are endogenous antioxidant enzymes that play crucial roles in scavenging oxygen free radicals and protecting cells from oxidative damage (Chen et al., 2017; Zhang & Yuan, 2006). SOD activity decreases with age and correlates positively with species lifespan. MAO serves as a marker of aging, with its content and activity positively correlated with the aging process (Ma, 2010; Wang, 2010). NO acts as an important intercellular and intracellular messenger and neurotransmitter, mediating neuronal responses to excitatory amino acids and enhancing learning and memory, though its synthesis decreases with age in the central nervous system.

This study employed a D-galactose-induced aging mouse model to investigate the effects and mechanisms of different *S. apetala* fruit extracts on learning and memory. The results demonstrated that all extracts effectively improved learning and memory performance, as evidenced by shortened escape latencies and prolonged target quadrant dwell times in the Morris water maze test ($P < 0.05$), while alleviating age-related brain atrophy and neuronal death. Biochemical analysis revealed that *S. apetala* fruit extracts enhanced brain SOD and GSH-Px activities and reduced NO content and MAO activity ($P < 0.05$). These findings suggest that *S. apetala* fruit extracts improve learning and memory in D-galactose-induced aging mice by attenuating neuronal damage, enhancing endogenous antioxidant enzyme activity, and reducing NO content and MAO activity in the brain.

The crude extracts of *S. apetala* fruit contain phenolic compounds such as total phenolics and anthocyanins. As plant secondary metabolites, phenolic compounds have become research hotspots in medicine, food, and chemical industries due to their antioxidant, antimicrobial, and anti-aging activities. Cao et al. (2015) isolated and identified eight phenolic acid monomers from fresh *S. apetala* fruits. Therefore, we hypothesize that these phenolic acid components are responsible for the observed improvements in learning and memory. However, further research is needed to identify the specific phenolic acid compounds that exert the primary effects.

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*Note: Figure translations are in progress. See original paper for figures.*